

N-SUBSTITUTED 3-HYDROXY-4-PYRIDINONES AND PHARMACEUTICALS
CONTAINING THEREOF

This application claims the benefit of priority of
5 U.S. Provisional Application No. 60/354,339 filed
February 5, 2002, hereby incorporated by reference.

Field of the Invention

This invention relates to novel N-substituted 3-
hydroxy-4-pyridinones and metal chelates, methods of
10 preparing N-substituted 3-hydroxy-4-pyridinones and metal
chelates, and pharmaceutical compositions containing new
N-substituted 3-hydroxy-4-pyridinones and/or their metal
chelates. This invention also relates to the use of N-
substituted 3-hydroxy-4-pyridinones and their metal
15 chelates as pharmaceutical agents for the treatment of
diseases, such as parasitic and viral infections,
conditions associated with inflammation and infection,
and conditions mediated by cell-proliferation or collagen
formation. This invention particularly relates to the N-
20 substituted 3-hydroxy-4-pyridinones as chelators for
chelation therapy of iron overload. This invention also
relates to the use metal chelates of N-substituted 3-
hydroxy-4-pyridinones as NMR contrast agents or
radiopharmaceuticals.

25

BACKGROUND OF THE INVENTION:

There are a number of inherited diseases, which are
associated with the gradual accumulation of iron. These
include β -thalassaemia major and thalassaemia intermedia.
30 Due to its facile redox chemistry, excess iron in human
body often results in irreversible damage to endocrine
organs and lethal cardiac toxicity. In humans such excess
iron can not be excreted via normal routes, namely, the
urine and the bile, and consequently chelation therapy is

essential (*J. Med. Chem.* **1998**, 41: 3347-3359; *Inorg. Chem. Acta* **1999**, 291: 238-246).

The objectives of iron-chelation therapy for iron overload are two fold: first, to produce negative iron balance by removing excess body iron; and second, to detoxify the excess iron while, and until, the first objective is achieved (*Drug Safety* **1997**, 17: 407-421). In order to be considered harmless, iron must be fully coordinated. If any of its six coordination sites remain uncoordinated, iron will participate in Fenton reactions, resulting in lipid peroxidation with organelle and cell damage from hydroxyl radicals (*Baillieres Clin. Haematol.* **1989**, 2: 195-256). Therefore, the iron chelator has to be able to form the iron complex with extremely high stability. Specificity of iron binding over other metals (e.g., zinc and copper) is also necessary to avoid chelation of these metals, which are needed for normal physiological activities.

Ideally, an iron chelator should have a low degree of penetration into the central nerve system and should produce a high degree of extraction of iron from hepatic cells, where iron is present in high levels (*Drug Safety* **1997**, 17: 407-421; *Acta Haematol.* **1996**, 95: 6-12). A second constraint of chelator design is that iron must not be redistributed from liver to other parts (e.g., heart and joint) of the body where it may be harmful. This requires that the iron complex be extremely stable. For a chelator to be efficiently absorbed from the gut, the molecular weight of the chelator has to be about 400 Dalton.

There has been considerable interest in the design of orally active iron chelators over the last two decades and many high-affinity iron chelators have been prepared (*J. Med. Chem.* **1990**, 33: 1749-1755; *J. Med. Chem.* **1993**, 36: 2448-2458; *J. Med. Chem.* **1993**, 36: 2448-2458; *J. Med. Chem.* **1994**, 37: 461-466; *J. Med. Chem.* **1994**, 37: 93-98; *J.*

Med. Chem. **1998**, 41: 3347-3359; *Eur. J. Med. Chem.* **1999**, 34: 475-485; *J. Med. Chem.* **2000**, 43: 1467-1475, *J. Pharm. Pharmacol.* **2000**, 52: 263-272; *Bioorg. Med. Chem.* **2001**, 9: 563-573; *Bioorg. Med. Chem.* **2001**, 9: 3041-3047;

5 *Tetrahedron* **2001**, 57:3479-3486). As a result, 1,2-dimethyl-3-hydroxypyridin-4-one (DMHP, CP20, Deferiprone) has been selected as the clinical candidate for the treatment of iron overload. One of the problems with such an N-alkyl-3-hydroxypyridin-4-one is the ability of the
10 free ligand and the resulting iron complex to rapidly penetrate cell membranes and other biological barriers (*Drug Met. Disp.* **1992**, 20: 256-261). A second problem is that N-alkyl-3-hydroxypyridin-4-ones are rapidly
15 metabolized by glyceronidation of the 3-hydroxy group, which will lead to disappearance of iron-chelating properties of the molecule. Despite recent developments, there is a continuing need for new iron chelators, which have high binding affinity for iron and are able to accumulate in liver, the major storage organ in iron-
20 overload conditions.

For many years radical scavenging antioxidants have been successfully used to protect synthetic material and food products from degradating process of oxidation (*Cosmet. Sci. Technol. Ser.* **1997**, 16: 159-179). Radical
25 scavengers have been proposed as neuroprotective agents for the treatment of disorders known to involve oxidative stress, such as stroke, tramatic brain injury, spinal cord injury, cerebral tumor, subharrchnoid haemorrhage/cerebral vasospam, cerebral ischaemia, stroke, Alzheimers' disease,
30 Huntington's disease, Parkinson's disease, Friedrich ataxia, motor neuron disease or multiple sclerosis. However, the effectiveness of radical scavengers in reducing oxidative stress within living biological environment is often undermined by the continual
35 production of free radicals mediated by iron. Since Fe is involved in the production of toxic free radicals, several radical scavenger-conjugated 3-hydroxy-4-pyridinones have

been prepared and studied as potent inhibitors of lipid peroxidation and cell toxicity (*J. Med. Chem.* **2000**, 43: 2779-2782). Some display a superior neuroprotective activity compared to dual administration of the radical scavenger, di-*tert*-butylphenol, and the iron chelator, Deferiprone, demonstrating the synergistic effect between the radical scavenger and the iron chelator.

Vanadium compounds, *in vitro*, stimulate glucose uptake and inhibit lipid break down, in a manner remarkably reminiscent of insulin's effect. Vanadium chelates with organic chelators present ways to fine tune the effect of vanadium, thereby minimizing any adverse effects without sacrificing important therapeutic benefits. Many compounds have been proposed as "insulin mimetics". These include vanadium complexes of pyronates (*J. Med. Chem.* **1992**, 35: 1489-1491; *J. Am. Chem. Soc.* **1995**, 117: 12759-12770; *Can. J. Physiol. Pharmacol.* **1995**, 73: 55-64; *Can. J. Physiol. Pharmacol.* **1996**, 74: 1001-1009; *J. Inorg. Biochem.* **1997**, 68: 109-116;), pyridinates (*Transition Metal Chem.* **2001**, 26: 219-223), picolinate (*Inorg. Chem.* **1999**, 38: 2288-2297), and cysteine ester (*Inorg. Chim. Acta* **1980**, 46: 2288-L119-L125), and have been recently reviewed (*J. Chem. Soc., Dalton Trans.* **2000**, 2885-2892; *Coord. Chem. Rev.* **2001**, 219-221: 1033-1053).

For vanadium to be useful as an orally available insulin mimetic agent, it must be able to cross biological membranes, both for the initial absorption process and intracellular uptake. Therefore, the metal chelate must have low molecular weight, neutral charge, and a fair degree of resistance to hydrolysis. The lipophilicity of the metal chelates must be balanced with its hydrophilicity, and possess adequate thermodynamic stability. As bidentate chelators for the design of vanadium chelates useful as insulin enhancing agents, 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones are exemplary. Both 3-hydroxy-4-pyrones and 3-hydroxy-4-

pyridinones form stable and neutrally charged vanadium chelates, which have an optimal combination of water solubility, reasonable hydrolytic stability, and significant lipophilicity (*J. Chem. Soc., Dalton Trans.* **2000**, 2885-2892; *Coord. Chem. Rev.* **2001**, 219-221: 1033-1053).

N-Alkyl-3-hydroxy-4-pyridinones form very stable six-coordinated gadolinium chelates (*Inorg. Chim. Acta* **1992**, 191: 57-63), potentially useful as MRI contrast agents. They also form very stable Zn(II) and Tin(II) complexes, which are useful in dental care formulations (*Polyhedron* **2000**, 19, 129-135; *Inorg. Chem.* **2001**, 40, 4384-4388). In addition, ^{67}Ga , ^{111}In and $^{99\text{m}}\text{Tc}$ complexes of N-alkyl-3-hydroxy-4-pyridinones have been studied as potential radiopharmaceuticals either for imaging or for the radiolabeling of white blood cells (*Nucl. Med. Biol.* **1992**, 19: 327-335; *Nucl. Med. Biol.* **1993**, 20, 857-863; *Inorg. Chem.* **1994**, 33, 5607-5679; *J. Med. Chem.* **1996**, 39: 3659-3670; *Eur. J. Nucl. Med.* **1999**, 26: 1400-1406). Other potential applications for substituted 3-hydroxy-4-pyridinones also include their use for the treatment of overload of other metals (e.g., copper, zinc, aluminum and plutonium) present in the body in deleterious amounts, inflammatory disease (*J. Biol. Chem.* **1996**, 271: 7965-7972; *Bioorg. Med. Chem. Lett.* **2001**, 11: 2573-2575), atherosclerotic disease (*Neuroreport* **1999**, 10: 717-725), neoplastic disease, and thrombosis.

UK Patent No. 2 136 807 discloses the use of 3-hydroxy-4-pyridinones for the treatment of iron overload arising from various causes, particularly that arising from pathological conditions such as thalassaemia, sickle cell anaemia, asplatic anaemia, and idiopathic haemochromatosis, often through the treatment of the first three conditions by regular blood transfusions. In addition, 3-hydroxy-4-pyridinones are of interest for the treatment of pathological conditions where there may be an

excess of iron deposited at certain sites even though patients do not exhibit a general iron overload.

EP Patent No. EP0335745 A1 discloses a process for preparation of substituted 3-hydroxy-4-pyridinones. EP
5 Patent No. EP0768302A2 and UK Patent No. GB2 269 589A also disclose synthesis of N-substituted 3-hydroxy-4-pyridinones and pharmaceutical compositions containing thereof. The substituent at the N atom is an aliphatic hydrocarbon group.

10 U.S. Patent No. 5,256,676 discloses synthesis of N-substituted 3-hydroxy-4-pyridinones and a method for the treatment of a patient having a condition caused by an iron-dependent parasite which comprises administering to
15 that patient a therapeutically effective amount of N-substituted 3-hydroxy-4-pyridinones.

Proposals have been made in EP Patent No. EP 0316279A2 to modify the 3-hydroxy group of the 3-hydroxy-4-pyridinones to provide a pro-drug form, i.e., in the form of a drug which does not itself possess the desired
20 biological activity but which is converted in vivo to a drug which does. UK Patent No. 2 269 589 specifically discloses the use of substituted 3-hydroxy-4-pyridinones as chelating agents for the treatment of iron overload.

International Publication No. WO 98/54138 discloses
25 preparation of 3-hydroxy-4-pyridinones as orally active iron chelators and their pharmaceutical formulations. The substituent at the N atom contains an aliphatic hydrocarbon group substituted by a hydroxy group or a carboxylic acid ester, sulfonic acid ester or a C1-6-alkoxy or C7-10-alkoxy ether. International Publication
30 No. WO 98/01458 also discloses preparation of N-substituted 3-hydroxy-4-pyridinones as iron(III) chelators. The N-substituents are selected from polyhydroxycarbons, such as saccharides.

35 UK Patent No. GB2345058A, International Publication No. WO 99/23075 and European patent applications

EP1006108A1 and EP1006112A1 disclose preparation of N-substituted hydroxypyridinone derivatives as reactive oxygen species scavengers. The N-substituted hydroxypyridinone derivatives contain both ortho-
5 hydroxypyridinone and oxygenated aryl (including heteroaryl) functionalities, which possess the dual ability to chelate iron and scavenge reactive oxygen species. The N-substituted 3-hydroxy-4-pyridinone derivatives are particularly useful for the treatment of a
10 condition associated with oxidative stress, such as oxidative damage of the central nervous system or an acute or chronic neurological disorder such as traumatic brain injury, spinal cord injury, cerebral tumor, subarachnoid haemorrhage/cerebral vasospasm, cerebral ischaemia, stroke
15 (ischaemic or haemorrhagic), Alzheimers' disease, Huntington's disease, Parkinson's disease, Friedrich ataxia, motor neuron disease or multiple sclerosis.

U.S. Patent No. 6,046,219 and International Publication Nos. WO 96/22021, WO96/41639, and WO 99/30562
20 disclose the use of hydroxypyridinone derivatives useful for the treatment of fibroproliferative disorders by inhibiting protein hydroxylation. Inhibitors of protein hydroxylases (including aspartyl/asparaginyl hydroxylase, prolyl 4-hydroxylase, and deoxyhypusine hydroxylase) block
25 the biochemical events that are required for the formation of excessive fibrocellular scar tissue, and therefore have anti-fibroproliferative properties of clinic importance.

U.S. Patent No. 5,877,210 discloses a conjugate comprising an inhibitor of phosphotyrosine phosphatase
30 covalently conjugated to a specific binding partner for a cell surface receptor found on B cells, wherein the inhibitor of phosphotyrosine phosphatase is a compound comprising a metal chelate of an organic chelator selected from the group consist of (a) keto-enol tautomers with the
35 keto and enol groups on adjacent carbon atoms that form 5-membered chelate ring or (b) beta-diketones in which the two keto groups are separated by one carbon atom, that

form a 6-membered chelate ring. The metal chelates disclosed include V(IV), Cu(II) and Ga(III) complexes of hydroxypyridinones, hydroxymates and acetylacetone. The inhibitory activity of 3-hydroxy-4-pyridinones on
5 mammalian tyrosine hydroxylase has also been reported recently (*Biochem. Pharmacol.* **2001**, 61: 285-290).

International Publication No. WO 01/12168 discloses a pharmaceutical composition comprising an iron chelator and another virus-inhibiting compound for the treatment of
10 viral infection, in particular of the human immunodeficiency (HIV). The iron chelator is selected from the group of hydroxamates or hydroxypyridinones while the viral-inhibiting compound is selected from protease inhibitors or reverse transcriptase inhibitors.

15 U.S. Patent No. 6,294,152 discloses Fe(III) complexes of 3-hydroxy-4-pyridinones useful as MRI contrast agents. In all the cases, the N-substituent is a simple or substituted alkyl or aryl group.

International Publication No. WO 91/12822 discloses
20 preparation of Fe(III) and Mn(II) complexes of 3-hydroxy-4-pyridinones useful as MRI contrast agents. The substituents on the pyridinone ring are simple alkyl groups substituted with phosphonate or sulfonate groups.

U.S. Patent Nos. 5,527,790 and 5,866,563 disclose
25 vanadium compositions for the treatment of elevated blood sugar. Vanadium chelates disclosed include those containing hydroxamates, O-heterocycle-substituted phenolates, 3-hydroxy-4-pyrones, and N-substituted 3-hydroxy-4-pyridinates. In all the cases, the N-
30 substituent is a simple or substituted alkyl or aryl group.

U.S. Patent No. 6,232,340 discloses organovanadium complexes and pharmaceutical compositions containing hydroxyoxovanadium(V), μ -oxo dimeric vanadium(V), and cis-
35 dioxovanadium(V) complexes for the treatment of diseases

or disease states, including use as antiproliferative and/or antimetastatic agents.

International Publication No. WO 93/10822 discloses cationic $^{99m}\text{Tc}(\text{IV})$ complexes with N-substituted 3-hydroxy-4-pyridinones as diagnostic scintigraphic imaging agents. The N-atom is directly attached to a carbon atom from a simple or substituted alkyl or aryl group.

International Publication No. WO 00/16736 discloses an oral care composition containing antiplaque agents. The antiplaque agents are metal complexes of $\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$, $\text{Sn}(\text{II})$, $\text{Fe}(\text{II})$, or $\text{Fe}(\text{III})$ with a specific class of cyclic α -hydroxyketones, including 3-hydroxy-4-pyrones.

However, there remains a need for therapeutic agents with enhanced efficacy, solution stability, and optimal combination of lipophilicity and hydrophilicity. This invention is directed towards meeting this need.

SUMMARY OF THE INVENTION:

One aspect of this invention is to provide novel N-substituted 3-hydroxy-4-pyridinones and pharmaceutical compositions containing these new N-substituted 3-hydroxy-4-pyridinones useful for the treatment of overload of iron and other metals (for example copper, zinc, aluminum and plutonium) present in the body in deleterious amounts.

Another aspect of this invention is to provide a method for the preparation of new N-substituted 3-hydroxy-4-pyridinones.

Another aspect of invention is related to the use of pharmaceutical compositions containing new N-substituted 3-hydroxy-4-pyridinones for the treatment of diseases, such as parasitic and viral infections, conditions associated with inflammation and infection, and conditions mediated by collagen formation.

Another aspect of invention is related to metal chelates of N-substituted 3-hydroxy-4-pyridinones, methods of preparing metal chelates of new N-substituted 3-hydroxy-4-pyridinones.

5 Another aspect of invention is to provide pharmaceutical agents or compositions containing metal chelates of new N-substituted 3-hydroxy-4-pyridinones for the treatment of diseases, such as viral infections, conditions associated with inflammation and infection,
10 and conditions mediated by cell-proliferation or collagen formation.

Another aspect of invention is related to the use of metal chelates of new N-substituted 3-hydroxy-4-pyridinones as NMR contrast agents.

15 Another aspect of this invention is related to the use metal chelates of new N-substituted 3-hydroxy-4-pyridinones as diagnostic or therapeutic radiopharmaceuticals.

20 DETAILED DESCRIPTION OF THE INVENTION:

For the last two decades, a large number of N-substituted 3-hydroxy-4-pyridinones have been synthesized and studied as iron chelators for the treatment of iron overload (*J. Med. Chem.* **1998**, 41: 3347-3359; *Inorg. Chem. Acta* **1999**, 291: 238-246; *Drug Safety* **1997**, 17: 407-421; *J. Med. Chem.* **1990**, 33: 1749-1755; *J. Med. Chem.* **1993**, 36: 2448-2458; *J. Med. Chem.* **1993**, 36: 2448-2458; *J. Med. Chem.* **1994**, 37: 461-466; *J. Med. Chem.* **1994**, 37: 93-98; *J. Med. Chem.* **1998**, 41: 3347-3359; *Eur. J. Med. Chem.* **1999**,
25 34: 475-485; *J. Med. Chem.* **2000**, 43: 1467-1475, *J. Pharm. Pharmacol.* **2000**, 52: 263-272; *Bioorg. Med. Chem.* **2001**, 9: 563-573; *Bioorg. Med. Chem.* **2001**, 9: 3041-3047; *Tetrahedron* **2001**, 57:3479-3486). The N-substituted 3-hydroxy-4-pyridinones of this invention are unique in such
30 a way that the N-atom of the pyridinone ring is directly
35

connected to the N-atom of a dialkylamino or acylamido-N or sulfonylamido-N group rather than a simple or substituted alkyl group. As a result, the dialkylamino, acylamido-N and sulfonylamido-N group imparts increased hydrophilicity. Since the molecular weight of these new chelators is generally <400 Dalton, they are expected to be efficiently absorbed from the gut. The lipophilicity arising from aromatic substituents of the dialkylamino or acylamido-N or sulfonylamido-N group should result in accumulation of the new chelator in hepatic cells, where iron is present in high levels. Like other 3-hydroxy-4-pyridinones previously disclosed, the new chelators form iron complexes with high stability. Therefore, the new N-substituted 3-hydroxy-4-pyridinones of the invention have the potential to be used as pharmaceutical agents for the treatment of overload of iron and other metals (e.g., copper, zinc, aluminum and plutonium) present in the body in deleterious amounts.

The N-substituted 3-hydroxy-4-pyridinones of this invention also have the potential to be used in combination with other pharmaceutical agents for the treatment of diseases. For example, it is known that iron chelation can influence HIV replication by inhibiting DNA synthesis via inactivation of iron-dependent ribonucleotide reductase. It has been demonstrated that the combination use of a virus-inhibiting agent (such as a protease inhibitor or a reverse transcriptase inhibitor) with an iron chelator results in synergistic effect for the treatment of viral infection, in particular of the HIV, (International Publication No. WO 01/12168).

Alternatively, the N-substituted 3-hydroxy-4-pyridinones of this invention can be conjugated to a protease inhibitor or a reverse transcriptase inhibitor via a direct covalent bond or through a metabolically cleaveable linker. In this way, the bioconjugate is bifunctional: inactivation of iron-dependent

ribonucleotide reductase and inhibition of protease or reverse transcriptase, resulting in synergistic effect for the treatment of viral infection.

The transition metal ion-dependent formation of hydroxyl radical from hydrogen peroxide in the presence of a reducing agent such as superoxide or ascorbate at low concentration is an important mechanism of "oxidative stress" leading to irreversible cell and tissue damage (*Bioorg. Med. Chem. Lett.* **2001**, 11: 2573-2575). Metal-ion mediated oxidative stress has been attributed a role in inflammation, atherosclerosis and Alzheimer's disease. It has been reported that iron chelators function as antioxidants to decrease plaque and aggregate formation in neurodegenerative diseases and atherosclerosis (*J. Surg. Res.* **1997**, 73: 35-42; *Neuroreport* **1999**, 10: 77-85). Iron chelators with the radical scavenging capability are of great potential for the treatment of disorders known to involve oxidative stress, such as stroke, traumatic brain injury, spinal cord injury, cerebral tumor, subarachnoid haemorrhage/cerebral vasospasm, cerebral ischaemia, stroke, Alzheimer's disease, Huntington's disease, Parkinson's disease, or multiple sclerosis.

The N-arylsulfonylamido- and N-arylcarboxylamido-substituted 3-hydroxy-4-pyridinones of this invention are bifunctional with the 3-hydroxy-4-pyridinone moiety for iron chelation to inhibit Fe-mediated free radical formation and the aryl group (such as benzene, pyridine and thiophene) for radical scavenging by reacting with hydroxyl radicals. Aromatic compounds such as phenylalanine and phenols react rapidly with hydroxyl radicals (*Bioorg. Med. Chem. Lett.* **2001**, 11: 2573-2575). A synergistic neuroprotective activity has been reported for radical scavenger-conjugated 3-hydroxy-4-pyridinones (*J. Med. Chem.* **2000**, 43: 2779-2782).

Zinc- and iron-containing metalloproteins have been studied as possible targets for antiviral and anticancer

therapy (*Anticancer Res.* **2001**, *21*, 931-958; *Exp. Biol. Med.* **2001**, *226*, 665-673). Viral and cellular zinc finger proteins and iron containing proteins are involved in cell proliferation, neovascularization, apoptosis, and viral infection. Matrix metalloproteinases are zinc metalloenzymes involved in remodeling of extracellular matrix, and play an important role in cancer as well as in numerous other disease (*Drug Discovery Today* **2001**, *6*: 478-482; *Pathol. Oncol. Res.* **2001**, *7*: 14-23; *Molecular Medicine Today* **2000**, *6*: 149-156). It has been proposed that disruption of metalloproteins by iron and zinc chelators is a key factor in controlling viral and proliferative diseases (*Anticancer Res.* **2001**, *21*, 931-958).

Various hydroxamates have been synthesized and studied as metalloproteinase inhibitors (MMPIs) for the treatment of cancer and other diseases (*Chem. Rev.* **1999**, *99*: 2735-2776; *Oncology* **2001**, *15* (7, suppl.): 39-46; *J. National Can. Res.* **2001**, *93*: 178-193; *Current Med. Chem.* **2001**, *8*: 425-474; *Expert Opin. Ther. Patents* **2002**, *12*: 29-43). A recent U.S. patent (U.S. Patent No. 6,232,340) discloses vanadium(V) complexes of 3-hydroxy-4-pyrones and N-alkyl 3-hydroxy-4-pyridinones as anti-proliferative and antimetastatic agents. It is not clear if the anti-proliferative and antimetastatic activity is due to the vanadium(V) complexes or from the dissociated chelator (3-hydroxy-4-pyrones or 3-hydroxy-4-pyridinones). However, it is known that Deferiprone (1,2-dimethyl-3-hydroxy-4-pyridinone) binds Zn(II) with high affinity with $\text{Log } K_{\text{ZnL}/[\text{Zn}][\text{L}]} = 7.19$ (*Inorg. Chem. Acta* **1992**, *191*, 57-63), and form very stable bis-ligand Zn(II) complexes (*Polyhedron* **2000**, *19*, 129-135; *Inorg. Chem.* **2001**, *40*, 4384-4388). The $\text{Log } K_{\text{ZnL}/[\text{Zn}][\text{L}]}$ is about 100-fold higher than that of N-hydroxyacetamide ($\text{Log } K_{\text{ZnL}/[\text{Zn}][\text{L}]} < 5$), the second smallest hydroxamate. Due to the similarity of 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones to hydroxamates, one can envisage that the anti-

proliferative and antimetastatic activity of the reported vanadium(V) complexes is actually due to MMP inhibition by the dissociated 3-hydroxy-4-pyrones and 3-hydroxy-4-pyridinones. In this connection, the N-substituted 3-hydroxy-4-pyridinones of this invention have the potential as MMP inhibitors useful for the treatment of cancer and many other diseases.

Bidentate N-substituted 3-hydroxy-4-pyridinones of this invention have low molecular weight (<400 Dalton), and are expected to form neutral vanadium chelate with a fair degree of resistance to hydrolysis. The lipophilicity of the vanadium chelates can be tuned by varying substituents on both the pyridinone ring and arylsulfonylamido or arylcarboxylamido group. Like vanadium complexes previously described (*J. Chem. Soc., Dalton Trans.* **2000**, 2885-2892; *Coord. Chem. Rev.* **2001**, 219-221: 1033-1053), the vanadium complexes of new N-substituted 3-hydroxy-4-pyridinones of this invention have the potential to be used as insulin enhancing agents.

Nuclear magnetic resonance (NMR) is based on the absorption of radio-frequency energy by the magnetic moment of atomic nuclei in samples placed in a strong magnetic field. Magnetic resonance imaging (MRI) of the human body relies mainly on the detection of most abundant type of nuclei, the hydrogen in water (and to some extent, fat). For discrimination of healthy and diseased tissues, adequate contrast is essential. Such contrast depends not only on differences in water concentration, but also on the NMR relaxation times T_1 and T_2 , which in turn are related to local mobility and interactions. MRI has become a widely accepted imaging modality for a variety of diseases. The availability of MRI devices has led to the use of MRI for the diagnosis of disease states and other internal abnormalities. Compared to other imaging modalities, MRI provides superior spatial resolution in tissues, and is safe due to the absence of exposure to X-rays or gamma radiation.

The continued use and rapid development of MRI has stimulated interest in the development of MRI contrast agents. MRI contrast agents increase both $1/T_1$ and $1/T_2$ to varying degrees depending on their nature as well as the applied magnetic field, and are used to improve diagnosis of disease by changing tissue signal intensity. Most MRI contrast agents commercially available or under clinical investigations are metal chelates containing paramagnetic metal ions, such as Fe^{3+} , Gd^{3+} , and Mn^{2+} . Agents such as gadolinium chelates are best visualized using T_1 -weighted images since the percentage change in $1/T_1$ in tissue is much greater than that in $1/T_2$ (Caravan, P. et al. *Chem. Rev.* **1999**, 99, 2293-2352). Iron-oxide particles generally lead to a much larger increase in $1/T_2$ than in $1/T_1$ and are best seen with in T_2 -weighted scans. The metal chelates have proved to be exceptionally well-tolerated class of contrast media. In particular, gadolinium MRI contrast agents do not show any nephrotoxicity in contrast to iodinated contrast media for CT (Runge, V. M. *J. Magn. Reson. Imaging* **2000**, 12, 205-213).

There are three basic interactions between the metal ion and water molecules (U.S. Patent No. 6,294,152). In an inner-sphere interaction, water molecules bind to and exchange with the metal ion, for a very effective contact. In an outer-sphere interaction, all the coordination sites of the metal ion are occupied by chelator(s) so that the water molecules are affected only through translational diffusion past the paramagnetic metal center. In the second-sphere interaction, the metal ion is wrapped with a set of donor atoms of a chelator or chelators, which form strong hydrogen binding with surrounding water molecules.

Mn^{2+} ion, which may conveniently be used in the form of its salt or chelates, has been proposed as an MRI contrast agent due to the five unpaired electrons in its d-orbitals. Manganese chelates, such as Mn(DPDP)

(TeslascanTM, Nycomed Amersham PL), Mn(DTPA), Mn(EDTA), Mn(TPPS₄) and their derivatives, have been used as MRI contrast agents for detection of liver diseases, cancer, and cardiovascular diseases. Paramagnetic metal chelates are safe as MRI contrast agents due to limited presence of free metal ion in the blood stream. Unfortunately, metal chelates also demonstrate reduced solution relaxivity relative to free metal ions due to replacement of coordinated water molecules by a chelator. Unlike the free metal ion, these manganese chelates are not known to bind endogenous macromolecules such as albumin. As a consequence, the dosage for metal chelates is much higher than that for the free Mn²⁺ ion.

Manganese(II) chloride has been proposed as an MRI contrast agent using intravenous injection. Indeed, even at very low i.v. dosages (5-10 µM/kg) manganese has been found to be particularly effective as a contrast agent for imaging liver. However, manganese salts, when administered intravenously as a contrast agent, may be teratogenic at clinical doses, and are known to interfere with the normal functioning of the heart by replacing calcium in the calcium pump of the heart.

In order to reduce the direct effect on the heart, oral administration of manganese(II) chloride as a liver imaging agent has been proposed (U.S. Patent Nos. 5,525,326 and 5,716,598). This ensures the passage of the contrast agent through the liver before entering heart. Although orally administered manganese(II) chloride is not teratogenic, the absorption of manganese(II) chloride through the gut is poor. As a result, the dosage required for clinical efficacy is of the order of 200 µM/kg. Such a high dosage will result in adverse cardiac effects.

International Publication Nos. WO 96/05867 and WO 97/02842, and U.S. Patent Nos. 5,525,326 and 5,716,598 disclose a contrast media comprising a physiologically

tolerable manganese compound, an uptake promoter and a physiologically tolerable carrier or exipient, having a manganese concentration of at least 0.3 mM or being in a dosage unit form containing at least 0.3 mM manganese.

- 5 The uptake promoter is capable of enhancing manganese transport across the membranes of the gastrointestinal (GI) tract. Compounds which have been found to be suitable for use as uptake promoters include reducing compounds containing an α -hydroxy ketone ($-\text{C}(\text{OH})-\text{CO}-$)
10 group, acids containing α - and/or β -hydroxy or amino groups, as well as vitamin D. The preferred α -hydroxy ketones include ascorbic acid, kojic acid, gluconic acid and salicylic acid. The uptake promoters most likely act as weak chelators for Mn^{2+} to form a spectrum of Mn^{2+}
15 containing species, which have better GI uptake when administered orally. The disclosed contrast media are particularly suitable for imaging of the liver.

- Mn^{2+} has an ionic radius similar to that of Ca^{2+} , and is handled similarly in many biological systems (*Circ. Res.* **1980**, 47: 721-727). For example, Mn^{2+} is known to enter cardiac myocytes through voltage-gated calcium channels (*Brain Res.* **1990**, 510: 289-295; (*Cell Calcium* **1993**, 14: 33-44). Therefore, it has been proposed to use Mn^{2+} -containing contrast media to image heart (*Magnetic Resonance in Medicine* **2001**, 46: 884-890; International
25 Publication No. WO 96/05867, U.S. Patents Nos. 5,525,326 and 5,716,598). However, the use of a high dosage of Mn^{2+} often results in cardiac toxicity due to replacement of calcium by manganese. To avoid the problem, Mn^{2+} -
30 containing contrast media comprising a mixture of $\text{Mn}^{2+}/\text{Ca}^{2+}$ salts have been proposed (U.S. Patent No. 5,980,863). The disclosed counter anions include acetate, gluconate, gluceptate, or lactate. Ascorbic acid has been used as antioxidant to stabilize Mn^{2+} in
35 biological systems.

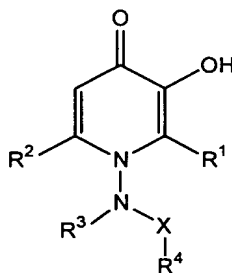
Fe^{3+} also has five unpaired electrons in its d-orbitals. Fe^{3+} metal ions, which interact with water

molecules by inner-sphere mechanism, are very effective for the enhancement of relaxation rate; but high dosage of the free metal ion often causes toxicity due to iron overload. The use of iron chelates reduces the toxicity; but the outer-sphere interaction is less effective in providing relaxation rate enhancement. International Publication No. WO 91/12822 discloses preparation of Fe^{3+} and Mn^{2+} complexes of 3-hydroxy-4-pyridinones useful as MRI contrast agents. U.S. Patent No. 6,294,152 discloses Fe^{3+} complexes of 3-hydroxy-4-pyridinones useful as MRI contrast agents. In all the cases, substituents on the pyridinone ring contain various hydrogen-binding functionalities, which are required for effective second-sphere interactions with surrounding water molecules, thereby enhancement of relaxation rate.

Synthesis of lanthanide complexes of 3-hydroxy-4-pyrones, including maltol and kojic acid, was previously reported (*J. Inorg. Nucl. Chem.* **1975**, 37: 1801-1802). The lanthanide, particularly Gd^{3+} , chelates of N-substituted 3-hydroxy-4-pyridinones of this invention are useful as MRI contrast agents via inner-sphere mechanism. Due to their large size, the coordination numbers of lanthanide metal ions are typically 8 and 9. In solution, six coordination sites of Gd^{3+} are occupied by three bidentate N-substituted 3-hydroxy-4-pyridinones while the remaining sites are available for water molecules to provide relaxation enhancement. Fe^{3+} and Mn^{2+} chelates of N-substituted 3-hydroxy-4-pyridinones are useful as MRI contrast agents via outer-sphere or second-sphere mechanism since they contain a dialkylamino or acylamido or sulfonylamido group, the nitrogen- or oxygen-heteroatoms of which can be used to form strong hydrogen bonds with surrounding water molecules. On the other hand, Fe^{3+} and Mn^{2+} chelates can be partially dissociated in biological systems to form Fe^{3+} and Mn^{2+} containing species, which may interact with water molecules via an inner-sphere mechanism.

The Ga-67, Tc-99m and In-111 chelates of N-substituted 3-hydroxy-4-pyridinones of this invention are useful as diagnostic radiopharmaceuticals for scintigraphic imaging. Tc-99m complexes of N-alkyl-3-hydroxypyridin-4-ones have been studied as potential radiopharmaceuticals for imaging kidney (*Nucl. Med. Biol.* **1993**, 20, 857-863; *Inorg. Chem.* **1994**, 33, 5607-5679) while Ga-67 complexes for imaging heart (*Nucl. Med. Biol.* **1992**, 19: 327-335). In-111 complexes of N-alkyl-3-hydroxypyridin-4-ones have been used for white blood cell labeling (*J. Med. Chem.* **1996**, 39: 3659-3670; *Eur. J. Nucl. Med.* **1999**, 26: 1400-1406).

According to one embodiment (1) of the invention, an N-substituted 3-hydroxy-4-pyridinone compound is provided, having the following formula:



and a pharmaceutically acceptable salt thereof, wherein:

X is selected from the group: CH₂, C(O), C(S), P(O)R³R⁴, SO₂, C(=NH)NH, C(O)NH, and C(S)NH;

R¹ and R² are independently selected from: H, C₁-C₁₀ alkyl substituted with 0-5 R⁵, C₂-C₁₀ alkenyl substituted with 0-5 R⁵, aryl substituted with 0-3 R⁵, and heteroaryl substituted with 0-3 R⁵;

R³ and R⁴ are independently selected from: C₁-C₁₀ alkyl substituted with 0-5 R⁵, C₂-C₁₀ alkenyl substituted with 0-5 R⁵, aryl substituted with 0-3 R⁵, heteroaryl substituted with 0-3 R⁵, or R³ and R⁴

may be taken together to form a C₅-C₇ cyclic alkyl group optionally interrupted with O or NR⁶;

R⁵ is elected from: OH, C(=O)R⁶, C(=O)OR⁶, C(=O)NR⁶R⁷, PO(OR⁶)(OR⁷), S(O)₂OR⁶;

5 R⁶ and R⁷ are independently selected from: H, C₁-C₁₀ alkyl, or aryl.

Another embodiment (2) of the invention is a compound according to embodiment (1) wherein:

X is selected from the group: CH₂, C(O), and SO₂;

10 R¹ and R² are independently selected from: H, C₁-C₃ alkyl substituted with 0-2 R⁵, and C₂-C₃ alkenyl substituted with 0-2 R⁵;

R³ and R⁴ are independently selected from: C₁-C₆ alkyl substituted with 0-3 R⁵, C₂-C₆ alkenyl substituted with 0-3 R⁵, aryl substituted with 0-3 R⁵, heteroaryl substituted with 0-3 R⁵, or R³ and R⁴ may be taken together to form a C₅-C₇ cyclic alkyl group optionally interrupted with O or NR⁶;

R⁵ is elected from: OH, C(=O)OH, and C(=O)NR⁶R⁷;

20 R⁶ and R⁷ are independently selected from: H and C₁-C₆ alkyl.

Another embodiment (3) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

25 X is selected from the group: CH₂, C(O), and SO₂;

R¹ is H;

R² is methyl or ethyl group;

R³ and R⁴ are independently selected from: aryl, heteroaryl, or R³ and R⁴ may be taken together form
30 a 5-7 membered cyclic alkyl.

Another embodiment (4) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

X is CH₂;

R¹ is H;

R² is methyl;

5 R³ and R⁴ are taken together form a 6-membered
cyclic piperidine ring.

Another embodiment (5) of the invention is a
compound according to any one of embodiments (1) to (2)
wherein:

X is CH₂;

10 R¹ is H;

R² is methyl;

R³ and R⁴ are taken together form a 6-membered cyclic
morphine ring.

Another embodiment (6) of the invention is a
15 compound according to any one of embodiments (1) to (2)
wherein:

X is CH₂;

R¹ is H;

R² is ethyl;

20 R³ and R⁴ are taken together form a 6-membered cyclic
morphine ring.

Another embodiment (7) of the invention is a
compound according to any one of embodiments (1) to (2)
wherein:

25 X is C(O);

R¹ is H;

R² is methyl;

R³ is H;

R⁴ is phenyl.

Another embodiment (8) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

X is C(O);
5 R¹ is H;
R² is ethyl;
R³ is H;
R⁴ is phenyl.

Another embodiment (9) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

X is C(O);
R¹ is H;
R² is methyl;
15 R³ is H;
R⁴ is 3-pyridine.

Another embodiment (10) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

20 X is C(O);
R¹ is H;
R² is methyl;
R³ is H;
R⁴ is 4-pyridine.

25 Another embodiment (11) of the invention is a compound according to any one of embodiments (1) to (2), wherein:

X is C(O);
R¹ is H;
30 R² is ethyl;

R³ is H;

R⁴ is 2-thiophene.

Another embodiment (12) of the invention is a compound according to any one of embodiments (1) to (2) wherein:

X is SO₂;

R¹ is H;

R² is methyl;

R³ is H;

10 R⁴ is phenyl.

Another embodiment (13) of the invention is a method for the preparation of an N-substituted 3-hydroxy-4-pyridinone compound according to any one of embodiments (1) to (12).

15 Another embodiment (14) of the invention is a pharmaceutical composition comprising a therapeutic effective amount of an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12) for the treatment of iron overload.

20 Another embodiment (15) of the invention is a pharmaceutical composition comprising a therapeutic effective amount of an N-substituted 3-hydroxy-4-pyridinone compound according to any one of embodiments (1) to (12) and a therapeutic metal for the treatment of
25 diseases, such as parasitic and viral infections, conditions associated with inflammation and infection, and conditions mediated by collagen formation.

Another embodiment (16) of the invention is a radiopharmaceutical of the formula:

30
$$M(C_h)_n,$$

and pharmaceutically acceptable salt thereof, wherein:

M is a radionuclide selected from: ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{90}Y , ^{149}Pr , ^{153}Sm , ^{159}Gd , ^{166}Ho , ^{169}Yb , ^{177}Lu , ^{186}Re , and ^{188}Re ;

n is 2 or 3;

5 C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

Another embodiment (17) of the invention is a radiopharmaceutical according to embodiment (16) wherein:

10 M is a radionuclide selected from: ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$, and ^{111}In ;

n is 3;

C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

15 Another embodiment (18) of the invention is a radiopharmaceutical according to embodiment (16) wherein:

M is ^{111}In ;

n is 3;

C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

20 Another embodiment (19) of the invention is a radiopharmaceutical according to embodiment (16) wherein:

M is ^{111}In ;

n is 3;

25 C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiment (4).

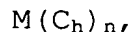
Another embodiment (20) of the invention is a radiopharmaceutical according to embodiment (16) wherein:

M is ^{111}In ;

n is 3;

30 C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiment (5).

Another embodiment (21) of the invention is an MRI contrast agent of the formula:



and pharmaceutically acceptable salt thereof, wherein:

5 M is a paramagnetic metal ion of atomic number 21-29, 42-44, or 58-70;

n is 2 or 3;

C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

10 Another embodiment (22) of the invention is an MRI contrast agent according to embodiment (21) wherein:

M is selected from: Fe³⁺ and Mn²⁺ and Gd³⁺;

n is 2 or 3;

15 C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

Another embodiment (23) of the invention is an MRI contrast agent according to embodiment (21) wherein:

M is Fe³⁺ and Mn²⁺;

n is 2 or 3;

20 C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(1).

Another embodiment (24) of the invention is an MRI contrast agent according to embodiment (21) wherein:

M is Fe³⁺;

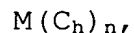
25 n is 3;

C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

30 Another embodiment (25) of the invention is a method of preparing a radiopharmaceutical of any one of embodiments (16) to (20).

Another embodiment (26) of the invention is a method of preparing an MRI contrast agent of any one of embodiments (21) to (24).

Another embodiment (27) of the invention is a
5 pharmaceutical composition comprising a metal chelate of the formula:



and pharmaceutically acceptable salt thereof, wherein:

10 M is a metal ion or a metal-containing core selected from: Ca^{2+} , Sn^{2+} , Cu^{2+} , Zn^{2+} , V^{3+} , $V^{5+}(O)$, or $V^{5+}(O)-O-V^{5+}(O)$;

n is 2 or 3;

C_h is an N-substituted 3-hydroxy-4-pyridinone according to embodiments (1)-(12).

15 Another embodiment (28) of the invention is a method of treating of a disease such as viral infections, conditions associated with inflammation and infection, and conditions mediated by cell-proliferation or collagen formation, comprising administering a patient in need of
20 such treatment a therapeutically effective amount of a pharmaceutical composition according to embodiment (27).

The compounds herein described may have asymmetric centers. Compounds of the invention containing an asymmetrically substituted atom may be isolated in
25 optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis from optically active starting materials. Many geometric isomers of olefins, C=N double bonds, and the like can
30 also be present in the compounds described herein, and all such stable isomers are contemplated in the invention. Cis and trans geometric isomers of the compounds of the invention are described and may be isolated as a mixture of isomers or as separated isomeric
35 forms. All chiral, diastereomeric, racemic forms and all

geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated. All processes used to prepare compounds of the invention and intermediates made therein
5 are considered to be part of the invention.

The term "chelator" as used herein, means that any organic compound containing at least two donor atoms (e.g., oxygen, nitrogen, sulfur or phosphine). Donor atoms are used to form coordination bonds with metal
10 ions. The chelator can be bidentate, tridentate, tetradentate, pentadentate, hexadentate, heptadentate, or octadentate depending on the number of donor atoms bonding to the metal ion.

The term "chelate" as used herein, means that any
15 metal complex comprising a metal ion and at least one organic chelator. The metal chelate may contain one or more identical chelators, and it may also contain two (binary ligand system) or three (ternary ligand system) different chelators.

20 The solution stability of a metal chelate depends on the nature of metal ion, the nature of donor atoms, and the structure of the chelator or chelators. The term "stable" is meant to indicate a metal chelate that is sufficiently robust to remain in solution without
25 significant dissociation of chelator(s).

Synthesis of metal chelates can be achieved according to the literature. For example, vanadium(V) chelates of N-substituted 3-hydroxy-4-pyridinones are prepared following the procedure disclosed in U.S. Patent No.
30 6,232,340, oxovanadium(IV) chelates using the procedure disclosed in U.S. Patent Nos. 5,866,563 and 5,527,790; and vanadium(III) chelates by the procedure disclosed in International Publication No. WO 00/24730. Zn(II), Cu(II), Sn(II) chelates N-substituted 3-hydroxy-4-
35 pyridinones are prepared following the disclosed procedure (International Publication No. WO 00/16736; *Polyhedron*

2000, 19, 129-135; *Inorg. Chem.* **2001**, 40, 4384-4388).

Lanthanide chelates of N-substituted 3-hydroxypyridin-4-ones are synthesized according to the published procedure (*J. Inorg. Nucl. Chem.* **1975**, 37: 1801-1802); Fe(III)

5 chelates using the procedure disclosed in U.S. Patent No. 6,294,152; ^{99m}Tc chelates by procedures described in published literatures (*Nucl. Med. Biol.* **1993**, 20, 857-863; *Inorg. Chem.* **1994**, 33, 5607-5679); and ^{111}In chelates using published procedures (*J. Med. Chem.* **1996**, 39: 3659-3670; 10 *Eur. J. Nucl. Med.* **1999**, 26: 1400-1406). Mn^{2+} -containing contrast media can be manufactured according to procedures described in International Publication No. WO 96/05867, U.S. Patent Nos. 5,525,326 and 5,716,598.

In general, a metal chelate, $\text{M}(\text{C}_h)_n$, of an N- 15 substituted 3-hydroxypyridin-4-one is prepared by reacting the metal salt with excess chelator in the presence of a base, for example sodium hydroxide in the aqueous medium or triethylamine in a non-aqueous media like ether, methanol, ethanol, isopropanol, or 20 acetonitrile. The $\text{M}:\text{C}_h$ ratio can be variable from 1:2 to 1:10. The preferred $\text{M}:\text{C}_h$ ratio is 1:2 for the metal chelate $\text{M}(\text{C}_h)_2$ and 1:3 for the metal chelate $\text{M}(\text{C}_h)_3$. The metal chelate is usually isolated from the reaction mixture in a solid form, and is characterized with a 25 variety of analytical methods (HPLC, LC-MS, IR, NMR, and x-ray crystallography). The metal chelate should have sufficient chemical purity for pharmaceutical applications, and is sufficiently robust to remain in solution without significant dissociation of the 30 chelator. For Mn^{2+} -based MRI contrast media, it may not be necessary to isolate the Mn-chelate from the mixture. In addition to large excess of chelator, calcium salt of the chelator is often added to reduce cardiac toxicity. The $\text{Mn}^{2+}:\text{C}_h$ ratio is up to 1:20 while the $\text{Mn}^{2+}:\text{Ca}^{2+}$ ratio 35 is up to 1:10. In addition, the pharmaceutical composition and contrast media may contain a lyophilization aid, including but are not limited to

mannitol, lactose, sorbitol, dextran, Ficoll, or
polyvinylpyrrolidine (PVP), a solubilization aid, such as
ethanol, glycerin, polyethylene glycol, or Pluronics, and
a bacteriostat, such as benzyl alcohol or benzalkonium
5 chloride.

The term "substituted," as used herein, means that
any one or more hydrogens on the designated atom is
replaced with a selection from the indicated group,
provided that the designated atom's normal valency is not
10 exceeded, and that the substitution results in a stable
compound. When a substituent is keto (i.e., =O), then 2
hydrogens on the atom are replaced. Keto substituents
are not present on aromatic moieties. When a ring system
(e.g., carbocyclic or heterocyclic) is said to be
15 substituted with a carbonyl group or a double bond, it is
intended that the carbonyl group or double bond be part
(i.e., within) of the ring.

The invention is intended to include all isotopes of
atoms occurring in the present compounds. Isotopes
20 include those atoms having the same atomic number but
different mass numbers. By way of general example and
without limitation, isotopes of hydrogen include tritium
and deuterium. Isotopes of carbon include C-13 and C-14.

When any variable (e.g., R^5) occurs more than one
25 time in any constituent or formula for a compound, its
definition at each occurrence is independent of its
definition at every other occurrence. Thus, for example,
if a group is shown to be substituted with 0-2 R^5 , then
said group may optionally be substituted with up to two
30 R^5 groups. Also, combinations of substituents and/or
variables are permissible only if such combinations
result in stable compounds.

When a bond to a substituent is shown to cross a
bond connecting two atoms in a ring, then such
35 substituent may be bonded to any atom on the ring. When
a substituent is listed without indicating the atom via

which such substituent is bonded to the rest of the compound of a given formula, then such substituent may be bonded via any atom in such substituent. Combinations of substituents and/or variables are permissible only if
5 such combinations result in stable compounds.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. Examples of alkyl include, but are not limited
10 to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, and s-pentyl. "Haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for
15 example $-C_vF_w$ where $v = 1$ to 3 and $w = 1$ to $(2v+1)$). Examples of haloalkyl include, but are not limited to, trifluoromethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl. "Alkoxy" represents an alkyl group as defined above with the indicated number of carbon atoms
20 attached through an oxygen bridge. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxy, and s-pentoxy. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl,
25 cyclobutyl, or cyclopentyl. "Alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl and propenyl. "Alkynyl"
30 is intended to include hydrocarbon chains of either a straight or branched configuration and one or more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl and propynyl.

"Halo" or "halogen" as used herein refers to fluoro,
35 chloro, bromo, and iodo; and "counterion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, and sulfate.

As used herein, "carbocycle" or "carbocyclic residue" is intended to mean any stable 3- to 7-membered monocyclic or bicyclic or 7-to 13-membered bicyclic or tricyclic, any of which may be saturated, partially
5 unsaturated, or aromatic. Examples of such carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane, [2.2.2]bicyclooctane, fluorenyl,
10 phenyl, naphthyl, indanyl, adamantyl, and tetrahydronaphthyl.

As used herein, the term "heterocycle" or "heterocyclic system" is intended to mean a stable 5-to 7-membered monocyclic or bicyclic or 7-to 10-membered
15 bicyclic heterocyclic ring which is saturated partially unsaturated or unsaturated (aromatic), and which consists of carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O and S and including any bicyclic group in which any of the above-
20 defined heterocyclic rings is fused to a benzene ring. The nitrogen and sulfur heteroatoms may optionally be oxidized. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The heterocyclic rings
25 described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. A nitrogen in the heterocycle may optionally be quaternized. It is preferred that when the total number of S and O atoms in the heterocycle exceeds 1, then these
30 heteroatoms are not adjacent to one another. It is preferred that the total number of S and O atoms in the heterocycle is not more than 1. As used herein, the term "aromatic heterocyclic system" or "heteroaryl" is
35 intended to mean a stable 5-to 7-membered monocyclic or bicyclic or 7-to 10-membered bicyclic heterocyclic aromatic ring which consists of carbon atoms and from 1 to 4 heterotams independently selected from the group.

consisting of N, O and S. It is preferred that the total number of S and O atoms in the aromatic heterocycle is not more than 1.

Examples of heterocycles include, but are not
 5 limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, 4aH-carbazolyl, carbolinyl,
 10 chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazoliny, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indoliziny, indolyl, 3H-indolyl,
 15 isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl,
 20 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl,
 25 pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinoliziny, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl,
 30 tetrahydroisoquinolinyl, tetrahydroquinolinyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl,
 35 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl. Preferred heterocycles include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl,

pyrazolyl, pyrrolidinyl, imidazolyl, indolyl,
benzimidazolyl, 1H-indazolyl, oxazolidinyl,
benzotriazolyl, benzisoxazolyl, oxindolyl,
benzoxazolinyl, and isatinoyl. Also included are fused
5 ring and spiro compounds containing, for example, the
above heterocycles.

The phrase "pharmaceutically acceptable" is employed
herein to refer to those compounds, materials,
compositions, and/or dosage forms which are, within the
10 scope of sound medical judgment, suitable for use in
contact with the tissues of human beings and animals
without excessive toxicity, irritation, allergic
response, or other problem or complication, commensurate
with a reasonable benefit/risk ratio.

15 As used herein, "pharmaceutically acceptable salts"
refer to derivatives of the disclosed compounds wherein
the parent compound is modified by making acid or base
salts thereof. Examples of pharmaceutically acceptable
salts include, but are not limited to, mineral or organic
20 acid salts of basic residues such as amines; and alkali
or organic salts of acidic residues such as carboxylic
acids. The pharmaceutically acceptable salts include the
conventional non-toxic salts or the quaternary ammonium
salts of the parent compound formed, for example, from
25 non-toxic inorganic or organic acids. For example, such
conventional non-toxic salts include those derived from
inorganic acids such as hydrochloric, hydrobromic,
sulfuric, sulfamic, phosphoric, and nitric; and the salts
prepared from organic acids such as acetic, propionic,
30 succinic, glycolic, stearic, lactic, malic, tartaric,
citric, ascorbic, pamoic, maleic, hydroxymaleic,
phenylacetic, glutamic, benzoic, salicylic, sulfanilic,
2-acetoxybenzoic, fumaric, toluenesulfonic,
methanesulfonic, ethane disulfonic, oxalic, and
35 isethionic.

The pharmaceutically acceptable salts of the invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared
5 by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or
10 acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

"Therapeutically-effective amount" refers to that
15 amount necessary to administer to a patient to achieve a therapeutic effect for the treatment of diseases, such as parasitic and viral infections, conditions associated with inflammation and infection, and conditions mediated by collagen formation. Methods of determining
20 therapeutically-effective amounts are well known to the skilled person.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a
25 reaction mixture, and formulation into an efficacious therapeutic agent.

Lyophilization aids useful in the preparation of diagnostic kits useful for the preparation of radiopharmaceuticals include but are not limited to
30 mannitol, lactose, sorbitol, dextran, Ficoll, and polyvinylpyrrolidone (PVP).

Solubilization aids useful in the preparation of radiopharmaceuticals and in diagnostic kits useful for the preparation of said radiopharmaceuticals include but
35 are not limited to ethanol, glycerin, polyethylene glycol, propylene glycol, polyoxyethylene sorbitan

monooleate, sorbitan monooleate, polysorbates, poly(oxyethylene)poly(oxypropylene)poly(oxyethylene) block copolymers (Pluronic) and lecithin. Preferred solubilizing aids are polyethylene glycol, and Pluronic.

5 Bacteriostats useful in the preparation of radiopharmaceuticals and in diagnostic kits useful for the preparation of said radiopharmaceuticals include but are not limited to benzyl alcohol, benzalkonium chloride, chlorbutanol, and methyl, propyl or butyl paraben.

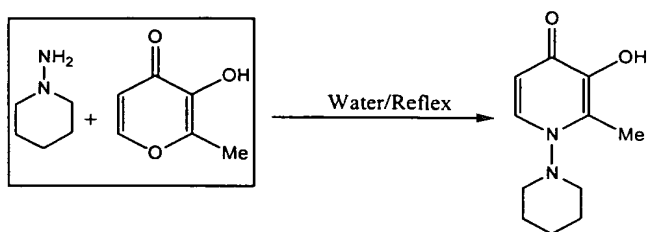
10

EXAMPLES:

Materials. 4-Aminomorphine, 1-aminopiperidine, ammonium chloride, benzoylhydrazine, ethylmaltol, isonicotinic acid hydrazide, maltol, nicotinic acid
15 hydrazide, phenylsulfonylhydrazide, sodium ascorbate, and thiophenecarboxylhydrazide were purchased from Aldrich, and were used as received. $^{111}\text{InCl}_3$ (in 0.05 N HCl) were purchased from NEN[®], N. Billerica, MA.

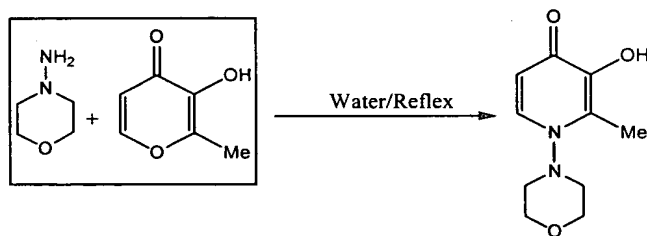
Instruments. ^1H NMR spectra were recorded on a 600
20 MHz Bruker spectrometer. The ^1H NMR data were reported as δ (ppm) relative to TMS. LC-MS spectra were collected using a HP1100 LC/MSD system with API-electrospray interface. The ITLC method used Gelman Sciences silica gel paper strips and a mixture of acetone and saline (1:1
25 = v:v) as eluant. By this method, the radiolabeled compound migrates to the solvent front while unchelated ^{111}In remain at the origin.

Example I - Synthesis of N-(1-Piperidinyl)-2-Methyl-3-
30 Hydroxy-4-Pyridinone (PMHP)



Maltol (3.6 g, 30 mmol) and 1-aminopiperidine (4.5 g, 45 mmol) were suspended in 100 mL of water. The mixture was heated to reflux for 2 days. The mixture was cooled to room temperature, and the solvent was removed under vacuum to give a dark brown liquid. The residue was allowed to stand overnight, a solid was formed. The solid was collected by filtration and was then recrystallized in water to give brownish microcrystals. The product was collected by filtration, washed with water, and dried under vacuum overnight. The yield was 0.64 g (~10.2%). LC-MS: $M/z = 209.3$ for $[C_{11}H_{16}N_2O_2]^+$. 1H NMR (600 MHz, in CD_3OD , chemical shift in ppm relative to TMS): 1.34 (m, 1H, CH_2); 1.81 (m, 5H, CH_2); 2.40 (s, 3H, CH_3); 3.00 (m, 4H, CH_2); 6.43 (d, 1H, $J_{HH} = 7.5$ MHz, CH_2 /pyridinone); and 7.99 (d, 1H, $J_{HH} = 7.5$ MHz, CH/pyridinone).

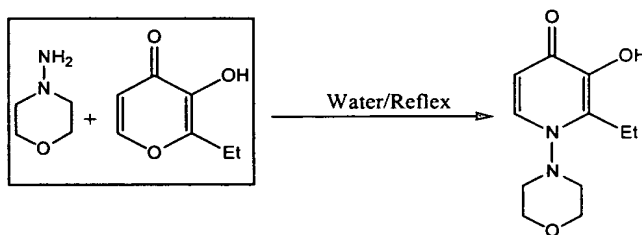
Example II - Synthesis of N-(1-Morphinyl)-2-Methyl-3-Hydroxy-4-Pyridinone (MMHP)



Maltol (3.6 g, 30 mmol) and 1-aminomorpholine (4.5 g, 45 mmol) were suspended in 100 mL of water. The mixture was heated to reflux for 2 days to give a dark brown solution. The solvent was removed under vacuum to give a black residue. Upon standing at room temperature

overnight, a solid was formed. The solid was collected by filtration and was then recrystallized in a mixture of water/methanol (2:1=v:v) to give brownish solid. The product was collected by filtration, washed with cold methanol, and dried under vacuum overnight. The yield was 0.60 g (~9.5%). LC-MS: $M/z = 211.2$ for $[C_{10}H_{14}N_2O_3]^+$. 1H NMR (600 MHz, in CD_3OD , chemical shift in ppm relative to TMS): 2.50 (s, 3H, CH_3); 3.00 (d, 2H, $J_{HH} = 10.9$ MHz, $CH_2/morphine$); 3.29 (m, 2H, $CH_2/morphine$); 3.79 (m, 2H, $CH_2/morphine$); 4.00 (d, 2H, $CH_2/morphine$); 6.53 (d, 1H, $J_{HH} = 7.5$ MHz, $CH_2/pyridinone$); and 8.09 (d, 1H, $J_{HH} = 7.5$ MHz, $CH/pyridinone$).

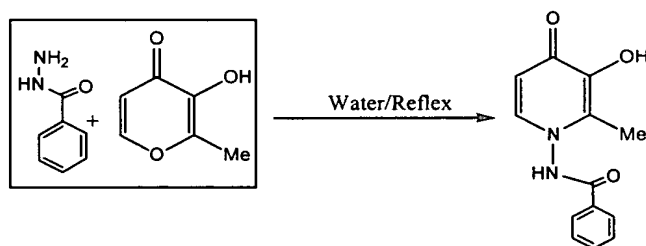
Example III - Synthesis of N-(1-Morphinyl)-2-Ethyl-3-Hydroxy-4-Pyridinone (MEHP)



To a round-bottom flask were added ethylmaltol (3.2 g, 24 mmol), 1-aminomorphine (4.0 g, 40 mmol) and 100 mL of water. The mixture was heated to reflux for 2 days to give a dark brown solution. Upon removal of the solvent, the dark residue was re-dissolved in a mixture of hot water/methanol (50%:50%=v:v) in the presence of charcoal. The mixture was filtered while hot. Solvents were removed under vacuum to give a black residue. After standing at room temperature for 2 days, a solid was formed. The solid was collected by filtration and was then recrystallized in a mixture of water/methanol (2:1=v:v) to give brownish microcrystals. The product was collected by filtration, washed with cold methanol, and dried under vacuum overnight. The yield was 0.48 g

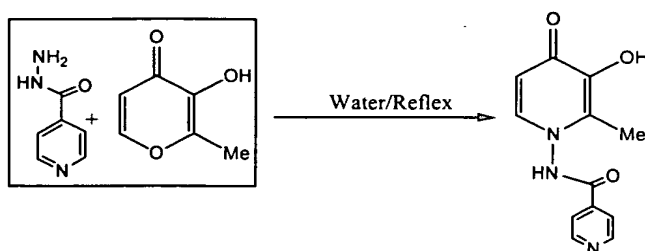
(~8.9%). LC-MS: $M/z = 225.3$ for $[C_{11}H_{16}N_2O_3]^+$. 1H NMR (600 MHz, in CD_3OD , chemical shift in ppm relative to TMS): 1.26 (t, 3H, CH_3); 2.95 (m, 4H, $J_{HH} = CH_2/ethyl$ and morphine); 3.33 (m, 2H, $CH_2/morphine$); 3.80 (m, 2H, $CH_2/morphine$); 4.00 (m, 2H, $CH_2/morphine$); 6.49 (d, 1H, $J_{HH} = 7.5$ mHz, CH/pyridinone); and 8.05 (d, 1H, $J_{HH} = 7.5$ mHz, CH/pyridinone).

Example IV - Synthesis of N-(benzoylamido)-2-Methyl-3-Hydroxy-4-Pyridinone (BMHP)



Maltol (12.5 g, 100 mmol) and benzoylhydrazine (14.5 g, 110 mmol) were suspended in 75 mL of water. The mixture was heated to reflux for 4 days, during which time a yellowish solid was formed. The mixture was filtered while hot to give 8.8 g of the product. The solid was collected and washed with hot methanol. The filtrate was evaporated under vacuum to almost dry and the residue was washed with hot methanol to give an additional 3.5 g of the product. The total yield was 12.3 g (~50%). LC-MS: $M/z = 245.2$ for $[C_{13}H_{12}N_2O_3]^+$. 1H NMR (600 MHz, in CD_3OD/CD_2Cl_2 (2:1), chemical shift in ppm relative to TMS): 2.37 (s, 3H, CH_3); 6.47 (d, 1H, $J_{HH} = 7.5$ mHz, CH/pyridinone); 7.55 - 8.00 (m, 5H, benzoyl); and 7.96 (d, 1H, $J_{HH} = 7.5$ mHz, CH/pyridinone).

Example V - Synthesis of N-(isonicotinylamido)-2-Methyl-3-Hydroxy-4-Pyridinone (IMHP)

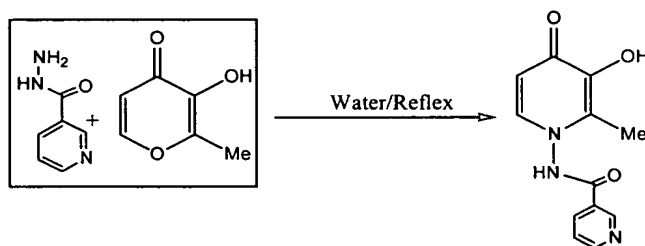


Maltol (3.6 g, 30 mmol) and isonicotinic acid hydrazide (4.35 g, 32 mmol) were suspended in 50 mL of water. The mixture was heated to reflux for 3 days.

- 5 Upon cooling to room temperature, the pale-yellow solid was separated by filtration, washed with methanol. The crude product was twice recrystallized from methanol. The overall yield was 2.75 g (~37%). LC-MS: $M/z = 246.3$ for $[C_{12}H_{11}N_3O_3]^+$. 1H NMR (600 MHz, in $D_2O/NaOD$, chemical shift in ppm relative to TMS): 2.30 (s, 3H, CH_3); 6.51 (d, 1H, $J_{HH} = 7.5$ MHz, CH/pyridinone); 7.25 (d, 1H, $J_{HH} = 7.5$ MHz, CH/pyridinone); 7.88 (d, 2H, $J_{HH} = 16.5$ MHz, CH/pyridine); and 8.69 (d, 1H, $J_{HH} = 16.5$ MHz, CH/pyridine).

15

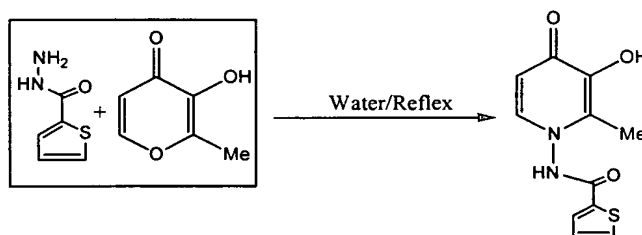
Example VI - Synthesis of N-(Nicotinylamido)-2-Methyl-3-Hydroxy-4-Pyridinone (NMHP)



- 20 Maltol (12.1 g, 100 mmol) and nicotinic acid hydrazide (14.2 g, 100 mmol) were suspended in 50 mL of water. The mixture was heated to reflux for 3 days. Upon cooling to room temperature, the pale-yellow solid was formed. The solid was separated by filtration, washed with methanol. The crude product was
- 25 recrystallized twice from methanol. The purified product

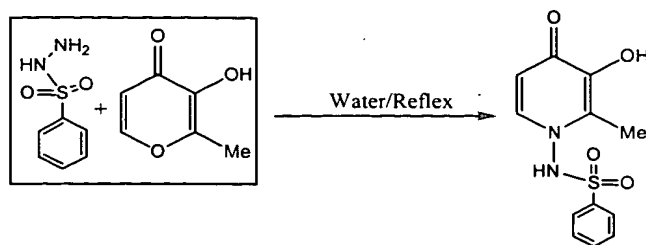
was dried under vacuum. The yield was 15.0 g (~61%).
 LC-MS: $M/z = 246.3$ for $[C_{12}H_{11}N_3O_3]^+$. 1H NMR (600 MHz, in $D_2O/NaOD$, chemical shift in ppm relative to TMS): 2.32 (s, 3H, CH_3); 6.52 (d, 1H, $J_{HH} = 7.5$ mHz, CH/pyridinone);
 5 7.28 (d, 1H, CH/pyridinone); 7.62 (m, 1H, CH/pyridine);
 8.35 (m, 1H, CH/pyridine); 8.70 (d, 1H, $J_{HH} = 5.0$ mHz, CH/pyridine); and 8.69 (s, 1H, CH/pyridine).

Example VII - Synthesis of N-(Thiophenecarboxylamido)-2-Methyl-3-Hydroxy-4-Pyridinone (TMHP)



Maltol (3.6 g, 30 mmol) and
 thiophenecarboxylhydrazide (4.26 g, 30 mmol) were
 15 suspended in 50 mL of water. The mixture was heated to
 reflux for 7 days, during which time a pale-yellow solid
 was formed. The solid was separated by filtration,
 washed with hot methanol. The crude product was
 recrystallized twice from methanol. The purified product
 20 was dried under vacuum overnight. The yield was 1.95 g
 (~26%). LC-MS: $M/z = 251.3$ for $[C_{11}H_{10}N_2O_3S]^+$. 1H NMR
 (600 MHz, in $D_2O/NaOD$, chemical shift in ppm relative to
 TMS): 2.28 (s, 3H, CH_3); 6.48 (m, 1H, $J_{HH} = 7.5$ mHz,
 CH/pyridinone); 7.26 (m, 2H, CH/pyridinone and
 25 thiophene); 7.64 (d, 1H, $J_{HH} = 5.0$ mHz, CH/thiophene); and
 7.71 (d, 1H, $J_{HH} = 3.7$ mHz, CH/thiophene).

Example VIII - Synthesis of N-(Phenylsulfonylamido)-2-Methyl-3-Hydroxy-4-Pyridinone (PSMHP)



Maltol (12.1 g, 100 mmol) and phenylsulfonhydrazide (17.2 g, 100 mmol) were suspended in 100 mL of water. The mixture was heated to reflux for 2 days. After cooling to room temperature the semi-solid residue was separated, and washed with hot methanol. The white solid was separated by filtration. The crude product was recrystallized from methanol. The purified product was dried under vacuum overnight. The yield was 2.15 g (~7.7%). LC-MS: $M/z = 281.2$ for $[C_{12}H_{12}N_2O_4S]^+$. 1H NMR (600 MHz, in $D_2O/NaOD$, chemical shift in ppm relative to TMS): 2.11 (s, 3H, CH_3); 6.26 (d, 1H, $J_{HH} = 7.1$ MHz, CH/pyridinone); 7.08 (d, 1H, $J_{HH} = 7.1$ MHz, CH/pyridinone); and 7.60-7.90 (m, 5H, phenyl).

Example IX - Synthesis of $^{111}In(PMHP)_3$

To a clean 5 mL vial were added 5 mg of PMHP, 1.5 mL of 0.5 M NH_4OAc (pH = 6.0), 0.5 mL of ethanol, and 4 μL of $^{111}InCl_3$ solution (~0.5 mCi) in 0.05 N HCl. The reaction mixture was allowed to stand at room temperature for ~20 min. The resulting solution was analyzed by an ITLC method using Gelman Sciences silicon gel paper strip, and a 50:50 mixture of saline and acetone as mobile phase. Using this method, $^{111}InCl_3$ and $[^{111}In]acetate$ remain at the origin while the radiolabeled $^{111}In(PMHP)_3$ migrates to the solvent front. The yield was 98%.

Example X - Synthesis of $^{111}In(MMHP)_3$

To a clean 5 mL vial were added 5 mg of MMHP, 1.5 mL of 0.5 M NH_4OAc (pH = 6.0), 0.5 mL of ethanol, and 4 μL of

$^{111}\text{InCl}_3$ solution (~0.5 mCi) in 0.05 N HCl. The reaction mixture was allowed to stand at room temperature for ~20 min. The resulting solution was analyzed by an ITLC method using Gelman Sciences silicon gel paper strip, and a 50:50 mixture of saline and acetone as mobile phase. Using this method, $^{111}\text{InCl}_3$ and [^{111}In]acetate remain at the origin while the radiolabeled $^{111}\text{In}(\text{MMHP})_3$ migrates to the solvent front. The yield was 99%.

10 Utility

The diagnostic radiopharmaceuticals are administered by intravenous injection, usually in saline solution, at a dose of 1 to 100 mCi per 70 kg body weight, or preferably at a dose of 5 to 50 mCi. Imaging is performed using known procedures.

The therapeutic radiopharmaceuticals are administered by intravenous injection, usually in saline solution, at a dose of 0.1 to 100 mCi per 70 kg body weight, or preferably at a dose of 0.5 to 5 mCi per 70 kg body weight.

The magnetic resonance imaging contrast agents of the invention may be used in a similar manner as other MRI agents as described in U.S. Patent 5,155,215; U.S. Patent No. 5,087,440; Margerstadt et al., Magn. Reson. Med., 1986, 3, 808; Runge et al., Radiology, 1988, 166, 835; and Bousquet et al., Radiology, 1988, 166, 693. Generally, sterile aqueous solutions of the contrast agents are administered to a patient intravenously in dosages ranging from 0.01 to 1.0 mmoles per kg body weight.

Oncomouse® Imaging

The study involves the use of the c-Neu Oncomouse® and FVB mice simultaneously as controls. The mice are anesthetized with sodium pentobarbital and injected with

approximately 0.5 mCi of radiopharmaceutical. Prior to injection, the tumor locations on each Oncomouse® are recorded and tumor size measured using calipers. The animals are positioned on the camera head so as to image the anterior or posterior of the animals. 5 Minute dynamic images are acquired serially over 2 hours using a 256x256 matrix and a zoom of 2x. Upon completion of the study, the images are evaluated by circumscribing the tumor as the target region of interest (ROI) and a background site in the neck area below the carotid salivary glands.

This model can be used to assess the effectiveness of the radiopharmaceuticals of the invention comprised of a beta, alpha or Auger electron emitting isotope. The radiopharmaceuticals are administered in appropriate amounts and the uptake in the tumors can be quantified either non-invasively by imaging for those isotopes with a coincident imageable gamma emission, or by excision of the tumors and counting the amount of radioactivity present by standard techniques. The therapeutic effect of radiopharmaceuticals can be assessed by monitoring the rate of growth of the tumors in control mice versus those in the mice administered the radiopharmaceuticals of the invention.

25

Rabbit Matrigel Model

This model was adapted from a matrigel model intended for the study of angiogenesis in mice. Matrigel (Becton & Dickinson, USA) is a basement membrane rich in laminin, collagen IV, entactin, HSPG and other growth factors. When combined with growth factors such as bFGF [500 ng/ml] or VEGF [2 µg/ml] and injected subcutaneously into the mid-abdominal region of the mice, it solidifies into a gel and stimulates angiogenesis at the site of injection within 4-8 days. In the rabbit model, New Zealand White rabbits (2.5-3.0 kg) are injected with 2.0

ml of matrigel, plus 1 μ g bFGF and 4 μ g VEGF. The radiopharmaceutical is then injected 7 days later and the images obtained.

This model can also be used to assess the effectiveness of radiopharmaceuticals of the invention comprised of a beta, alpha or Auger electron emitting isotope. The radiopharmaceuticals are administered in appropriate amounts and the uptake at the angiogenic sites can be quantified either non-invasively by imaging for those isotopes with a coincident imageable gamma emission, or by excision of the angiogenic sites and counting the amount of radioactivity present by standard techniques. The therapeutic effect of radiopharmaceuticals can be assessed by monitoring the rate of growth of the angiogenic sites in control rabbits versus those in the rabbits administered the radiopharmaceuticals of the invention.

Imaging of the laterals were for 2 hours with a 256x256 matrix, no zoom, 5 minute dynamic images. A known source is placed in the image field (20-90 μ Ci) to evaluate region of interest (ROI) uptake. Images were also acquired 24 hours post injection to determine retention of the compound in the tumor. The uptake is determined by taking the fraction of the total counts in an inscribed area for ROI/source and multiplying the known μ Ci. The result is μ Ci for the ROI.

This model can also be used to assess the effectiveness of the radiopharmaceuticals of the invention comprised of a beta, alpha or Auger electron emitting isotope. The radiopharmaceuticals are administered in appropriate amounts and the uptake in the tumors can be quantified either non-invasively by imaging for those isotopes with a coincident imageable gamma emission, or by excision of the tumors and counting the amount of radioactivity present by standard techniques.

The therapeutic effect of the radiopharmaceuticals can be assessed by monitoring the size of the tumors over time.

Obviously, numerous modifications and variations of the invention are possible in light of the above
5 teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.